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The effect of Cd on ultrastructure of wheat

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Abstract

Transmission Electron Microscope (TEM) was used to investigate the damage of wheat seedlings by cadmium and the distribution of Cd^{2+} in different parts of wheat seedlings was discussed. Experiments show that Cd is more mobile than other metals and the pollution damages the root tip cells. In addition, lots of abnormal symptoms were observed, including cell-wall thinning and cell deforming, cytoplasm and organelle swelling or even becoming vacuolization, nuclear membrane becoming disrupted and square cells being formed. The damage of Cd^{2+} on wheat leaves was less serious than that of root tips. Cd pollution may promote cell cleavage, resulting in swelling and disturbance of the layered structure of chloroplast, leading mitochondrion to swelling or even becoming round. The dense electron particles exist in ektexine, extracellular matrix, vacuolar of root cells as polluted by low concentration of Cd and exist outer the wall and inside the cytoplasm of leaves cells as polluted by different levels of Cd.

Key-words: Cd pollution; wheat seedling; damage; ultrastructure

1. Introduction

Heavy metal pollution in soil and irrigation water has become an important part of eco-pollution. Cd is regarded as one of the most threatening poisonous heavy metals for its high mobility and toxicity [1, 2].Adults can endure no more than 60-70μg of Cd according to World Health Organization standard. The maximum content of Cd according to FAO/WHO in rice is 0.1mg/kg [3, 4]. Metabolic disorders of plants may take place as stressed by Cd such as growth of root and the water and nutrient absorb hindered, photosynthetic and respiratory intensity decreased, hydration metabolic disorder[5-8]. Dramatic changes in ion and water homeostasis lead to molecular damage[9], growth inhibition or even death [10]. However, few reports of Cd^{2+} inference on ultrastructure of plants were found [11,12]. Aimed at providing a theory for physiology toxicity and genetic toxicity of Cd^{2+} on wheat seedlings, a hydroponical experiment was carried out to study damage mechanism of Cd^{2+} on ultrastructure of wheat seedlings root tips and leaves, which could also be used to address migration rule of Cd^{2+} in the soil-organism system.

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2. Materials and methods

2.1 Materials

Yan Nong 19' wheat, ubiquitous in Xuzhou area, was selected as experiment seedlings.

2.2 Methods

2.2.1. Wheat cultivation

Firstly, the fine and plump seeds were dipped in de-ionized water for twelve hours after surface sterilization by 5% NaClO and swilled several times by de-ionized water based on the rule for seed germination (GB5520-85). Then, a filter paper was dipped into culture dishes of 1200mm diameter with 10ml de-ionized water, and $CdCl_2$ solution of 0mg/L,0.5mg/L,30mg/L respectively. At last, the seeds were laid on filter papers uniformly with the embryo upturned and stored into the illumination plant box. The cultivated condition was set as follows: illumination lasting 12h, illumination intensity keeping at 36μmol·m^{-2} ·s^{-1}, temperature at 20 ℃ (daytime) or 15℃ (night), and moist condition.

2.2.2. Sample preparation and transmission electron microscopy

The taproot tips and the middle leaves were sampled and cut into segments of 1-2mm after wheat seedlings have buded for seven days. Samples were fixed by 2.5% of glutaraldehyde and 4% of osmic acid, dehydrated by increasing percent of ethanol and embedded by Epon812. Ultrathin sections of root and leaf samples were cut with LKB-Ⅴ microtome, mounted in copper grids and dyed with uranium acetate and plumbum citrate. The sections were observed by JEM-200cx Electron Microscope with accelerating voltage at 80kv, current at 50μA, condenser lens aperture of 200μm and objective aperture of 40μm.

3. Results

3.1 Damage to root-tip cells

Compared with the healthy cells (Fig.1 01), dysmorphic cell and slight ptlasmolysis happened in root-tip photobiont cells stressed by 0.5mg/L Cd^{2+} and the dark electron dense particles sedimentated on ektexine and endothecium and inner parts of the cell (Fig.1 A).The cell wall became thin with slight caniniform distortion, Moreover, many vesicles appeared in cytoplasm (Fig.1 B) with dark particles inside (Fig.1 C). The phenomena mentioned above show that even low concentration Cd has great influences on the wall of wheat root-tip cells and transmitting ability. More serious damages took place to root-tip cells of wheat that was grown in 30mg/L Cd^{2+} and squared cells appeared (Fig.1 D), which probably caused by cytoplasmic or organelles swelling and the wall pressure increasing. Swelling and even hollow organelles (Fig.1 E) and condensed chromatin and double nuclear membrane damage (Fig.1 F) were also observed

3.2 Damage to leaf Cells

In the healthy cells (Fig.2 03), the chloroplast adhered to the wall of the cell and wrapped by membrane, was shaped like a ellipse. Karyoplasms was uniformed, nuclear membrane was intact and mitochondria contained clear structure and distinct cristae. There was no significant difference in the structure of leaves cells grown in 0.5mg/L Cd^{2+} but a few vacuoles and more dividing cells existed and dark electron dense particles sedimentated both inside and outside of the walls (Fig.2 A). It can be deduced that cell division is promoted as stressed by low concentration of Cd^{2+} from increasing of the dry weight and fresh weight of wheat that grown in 0.5mg/L Cd^{2+}. In cells cultivated with 30mg/L Cd^{2+}, dark electron dense particles sedimentated on outer walls (Fig.2 B) , chloroplast swelled and stromal lamellae was disturbed, mitochondria swelled like a ball(Fig 2 C) The progress of cell damge was observed that cell nuclei disappeared at first, then the chloroplast disappeared, the electron dense of cytoplasm was diluted afterwards, and mitochondria swelled and eventually disappeared (Fig 2 D). The plasma membrane were exfoliated from the cell surface and some of the cytoplasm and organelle flowed between the wall and plasmalemma (Fig 2 E).

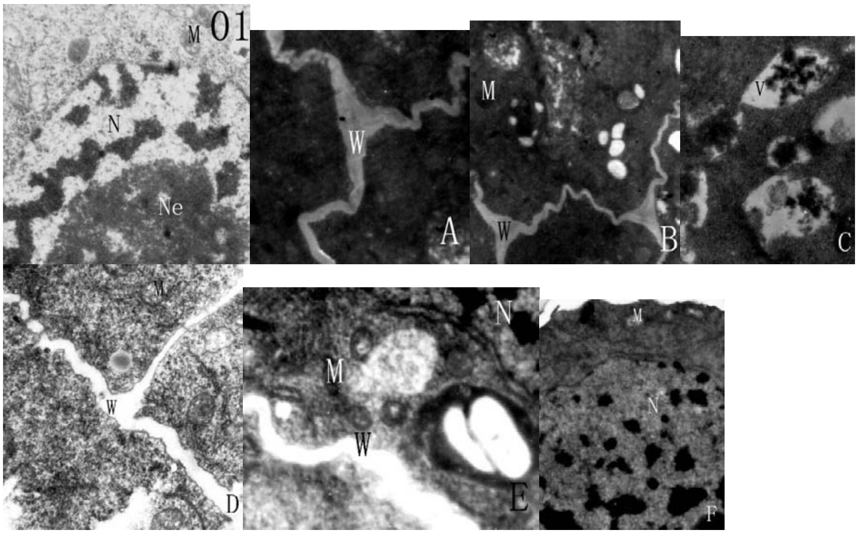

Fig. 1. Change of cell ultrastructure of Wheat root tips under Cd pollution

(01.Cell mitochondria and nucleus of healthy cell, ×16000; A. Plasmolysis and dark electron dense particles sedimentated on ektexine and endothecium of root-tip cells stressed by 0.5mg/L Cd^{2+}, ×8000; B. Wall distortion and Vesicles happened in cytoplasm stressed by 0.5mg/L Cd^{2+}, ×8000; C. Dark electron dense particles in vesicles stressed by 0.5mg/ LCd^{2+}, × 20000; D. Squared cells appeared, Stressed by 30mg/ L Cd^{2+}, × 20000; E. Swelling and even hollow organelles appeared, Stressed by 30mg/L Cd^{2+}, ×16000; F. Condensed chromatin and double nuclear membrane damaged, , Stressed by 30mg/L Cd^{2+}, ×20000; W. cell wall; M. mitochondria; N. nucleus; Nu. Nucleolus; V. vacuole; Ch. Chlorplast.

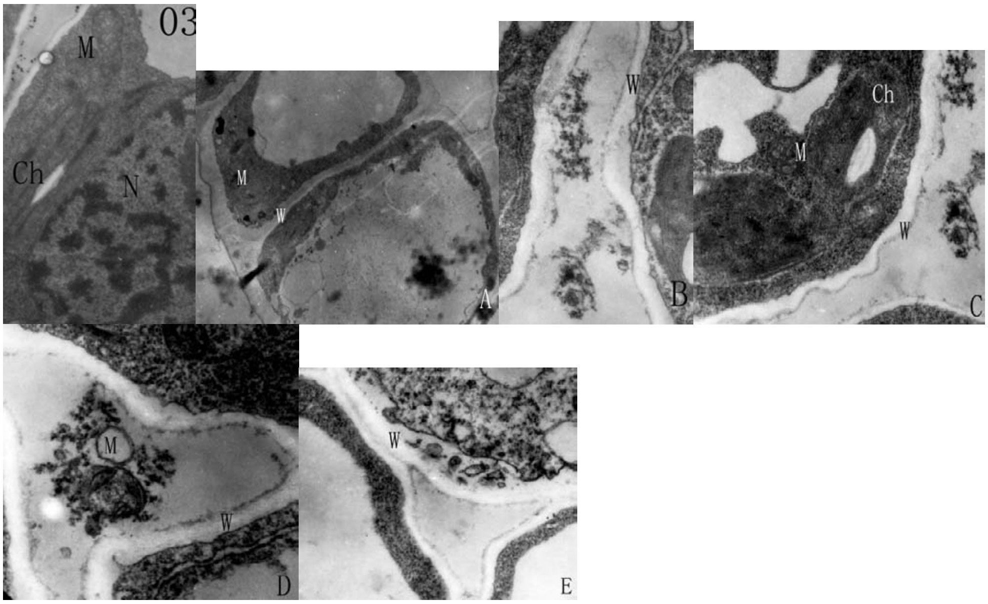

Fig. 2. Change of cell ultrastructure of wheet leaf under Cd pollution

(03. Control chloroplast, nucleus, mitochondria,×8000; A. Dividing cells and dark electron dense particles sedimentated both inside and outside of the walls stressed by 0.5mg/L Cd^{2+}, ×20000; B. Dark electron dense particles sedimentated outer walls stressed by 30mg/L

Cd^{2+}, ×8000; C. Swelling chloroplast and mitochondria stressed by 30mg/L Cd^{2+}, ×20000; D. Cell apoptosis stressed by 30mg/L Cd^{2+}, ×20000; E. Plasma membrane exfoliated stressed by 30mg/L Cd^{2+}, ×16000)

4. Discussion

4.1. Transplantation of Cd in cell walls of wheat seedlings

Cell wall is regarded as an important site for metal ion storage and may act as an excretory organ for heavy metals preventing heavy metal into protoplasm and keeping them from damage [9]. Electrons dense particles were founded in exine walls and extracellular matrix of root-tip and leaves cells, and less in exine walls of root-tip cells and more in leaves cells stressed by Cd^{2+} especially, which indicated that Cd^{2+}, has high transport capacity in wheat seedlings. It was founded that Cd^{2+},content in root was 7-20 times higher than that in leaves, which is higher than Zn content and less than Pb and Cu contents.

Cd mainly distributes in vacuolar and exine walls of the root-tip cells stressed by 0.5mg/L Cd^{2+}. Vacuoles can distill the heavy metal from the other substances because it is an independent area filled with some organic acid, organic alkali and protein that can bond with heavy metal and passivate it [13]. This is another biologic tolerance mechanis of wheat seedlings towards high dose of Cd

4.2. Damage of Cd to mitochondria of wheat seedlings

Mitochondrion, energy metabolism site of cell, is able to transform organic matter into the direct energy ATP. Because of the swelling of mitochondria crista, the functions of mitochondria decreases and even disappears which is also casued by heavy metal dismissing enzymes and resulting in insufficiency of protein material and depended endothecium membrane [14].

Cd, however, has less influence on mitochondrion. Little variation in mitochondrion of root-tip cells stressed by 0.5mg/L Cd^{2+} was observed. Generally, mitochondria was not vulnerable. And its cristae only swells and remodels as stressed by 30mg/L Cd^{2+}. During the progress of leaves cell apoptosis, mitochondria can bear the stress of 30mg/L Cd^{2+}, which is coincident with the previous study [15].

4.3. Damage of Cd to chloroplast of wheat seedlings

The chloroplast is the organelle for photosynthesis. Granum lamellae and stromal lamellae are the room of photosynthesis. The biggest light receiving and the most effective photosynthesis are provided by the normal and ordered chloroplast. The lamellae, acting as the skeleton of enzyme, makes light energy be captured highly and concentatively, which is helpful to form a persistent metabolizing carrier and promote absorption and transformation of light energy. The photosynthesis efficiency will be weakened if the structure of chloroplast is broken [14, 16]. Slight swelling of chloroplast has been found in the leaf cells stressed by 0.5mg/L Cd^{2+}. The swelling was fairly common in the cells stressed by 30mg/L Cd^{2+}. Some chloroplast membrane was demolished but the lamellae preserves original frame of chloroplast and maintains the basic function of chloroplast. Chloroplast was one of sensitive organelles in the progress of leaves cell apoptosis.

4.4. Damage of Cd to nucleus of wheat seedlings

The cell nucleus, in which all genetic information is stored, replicated and expressed controls the activities of cell . The structure of nucleus and nucleolus, the backbone of metabolic capability, is closely related to.the correct expression of genetic information and synthesis of ribosome [17]. It is revealed that low concentration of Cd has no effect on root-tip and leaves cells nucleolus. Aggregated and geatinous karyoplasms, disrupted nuclear membrane occured in root-tip cells and disrupted nuclear membrane occured in leaves cells when they are stressed by 30mg/L Cd^{2+}. Therefore, cell nucleus was the most sensitive organelle of Cd stress in the progress of leaves cell apoptosis.

5. Conclusion

1) Cd has a high transport capacity in *YanLong19* wheat seedlings. Electrons dense particles were founded in exine walls and extracellular matrix of root-tip and leaves cells and less in exine walls of root-tip cells and more in leaves cells stressed by Cd^{2+} especially.

2) Cd has little influence on mitochondrion. Little variation occurs in root-tip mitochondrion stressed by 0.5mg/L Cd^{2+} . Swelling and remodeling of mitochondria cristae only takes place when it is stressed by 30mg/L Cd Cd^{2+}.

3) The progress of cell apoptosis is that cell nuclei disappear initially, then the chloroplast and the cytoplasm decrease, with that mitochondrial swells and eventually disappears..

Acknowledgements

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References

[1] W.D. Chen and H.G. yao, Scientia Agricultura Sinica, 38 (2005) 528-537.
[2] F.X. Liu and Y. Wu, .Journal of Agro-Environment Science, 26 (2007) 1860-1864.
[3] M. Wan, W. Zhou qand B. Lin, Scientia Agricultura Sinica, 36 (2003) 671-675.
[4] FAO/WHO. Joint committee on food additives and contaiminants. The Netherlands. 20-24 (1995) 32.
[5] A.B. Andrei, I.S. Vera and E.T. Viktor, Euphytica. 131 (2003) 25-35.
[6] N. Boussama, O. Ouariti and M.H. Ghorbal, Journal of Plant Nutrition. 22 (1999) 731-752.
[7] Z. Krupa, Plant Science. 138 (1998) 149-156.
[8] H.F. Chien, Plant Science. 15 (2000) 111-115.
[9] Y. Wang, J. Fang and S.S. Leonard, Free Radical Biology & Medicine. 36 (2004) 14342-14431.
[10] J. Zhang and W.S. Shu, Journal of Plant Physiology and Molecular Biology. 32 (2006) 1-8.
[11] Q.Y. Wu and J.C. Yang, Journal of Agro-Environment Science. 27 (2007) 2216-2220.
[12] X. Xiao and Q.Y. Feng, Micro-mechanism of Damage of Soluble Lead (Pb2+) on Wheat Seedlings Cells. Water-Rock Interaction. (2007) 1625-1628.
[13] W. Rauser, Plant Physiol. 109 (1995) 1141-1149.
[14] F.H. Chen, Y. Zheng, ACTA Scientiarum Naturalium Universitatis Sunyatseni. 44 (2005) 107-110.
[15] D.T.Yang, G.X. Shi and W.P. You, Journal of Nanjing Normal University. 23 (2000) 91-95.
[16] RS.Alberte, Plant Physiol. 59 (1997) 351-353.
[17] F. Van Assche and H. Clijsters, Plant Cell Environ. 13 (1990) 195-206.